

VIGET: A web portal for study of vaccine-induced host responses based on Reactome pathways and ImmPort data

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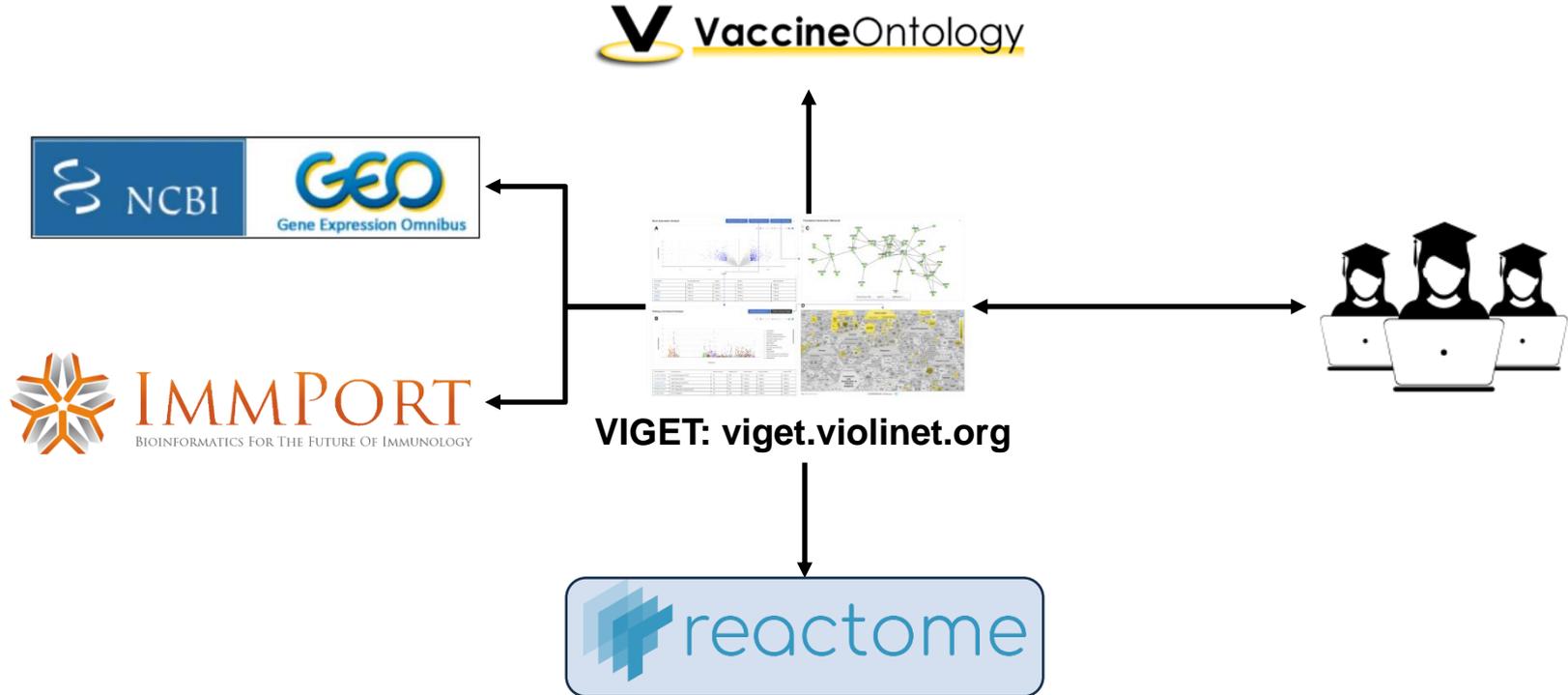
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Ann Arbor, Michigan*



Outline

- Introduction
- Development of VIGET
 - Data collection from ImmPort and GEO
 - Software Development
 - Major analysis and visualization features
- Live software demo
- Application cases

Project Goals



Reactome: an Open Source Pathway Knowledgebase



About Content Docs Tools Community Download

Find Reactions, Proteins and Pathways

e.g. O95631, NTN1, signaling by EGFR, glucose

Go!



Pathway Browser

Visualize and interact with Reactome biological pathways



Analysis Tools

Merges pathway identifier mapping, over-representation, and expression analysis



ReactomeFIViz

Designed to find pathways and network patterns related to cancer and other types of diseases



Documentation

Information to browse the database and use its principal tools for data analysis



Reactome Research Spotlight

The Zika virus (ZIKV) is an emergent arthropod-borne virus (arbovirus) responsible for congenital Zika syndrome (CZS) and a range of other congenital malformations. With little known about the pathways involved in CZS, Gratton et al in the February 2020 issue of *Microorganisms* conducted a meta-analysis of transcriptome studies to identify the genes and pathways altered during Zika infection. Reactome analysis identified interferon, pro-inflammatory, and chemokines signaling as well as apoptosis as key IFN signaling pathways in ZIKV-infected cells with three new candidate genes involved in hNPCs infection identified: APOL6, XAF1, and TNFRSF1.

ARCHIVE



Why Reactome

Reactome is a free, open-source, curated and peer-reviewed pathway database. Our goal is to provide intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education.



The development of Reactome is supported by grants from the US National Institutes of Health (U24 HG012198) and the European Molecular Biology Laboratory.



Latest News

V85 released

V84 released

Reactome is hiring!

Reactome named as a Global Core Biodata Resource

Version 83 Released

Reactome Research Spotlight

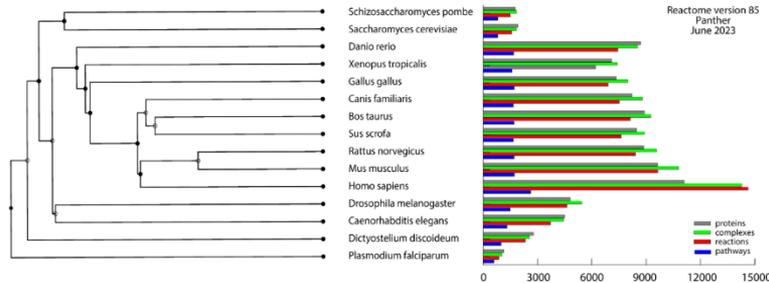
Collaboration with PharmGKB

Version 82 Released

<https://reactome.org>

Reactome: an Open Source Pathway Knowledgebase

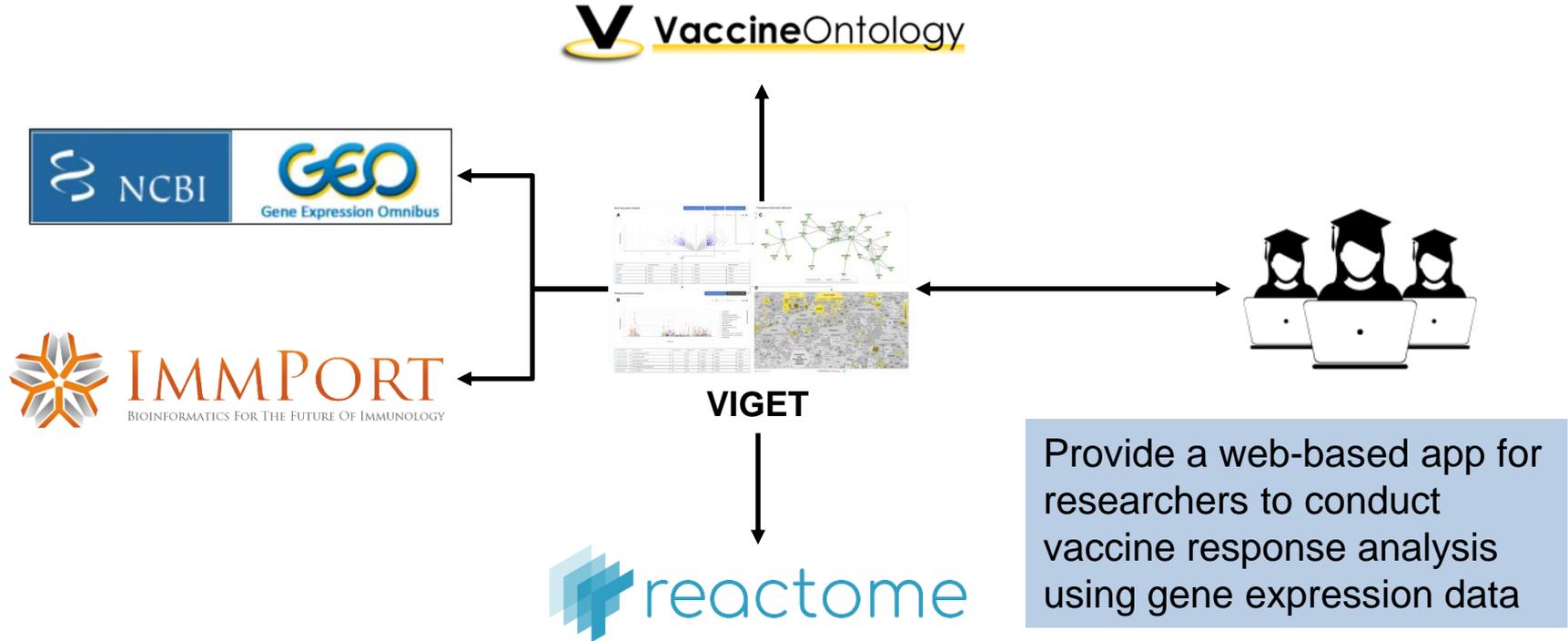
Statistics



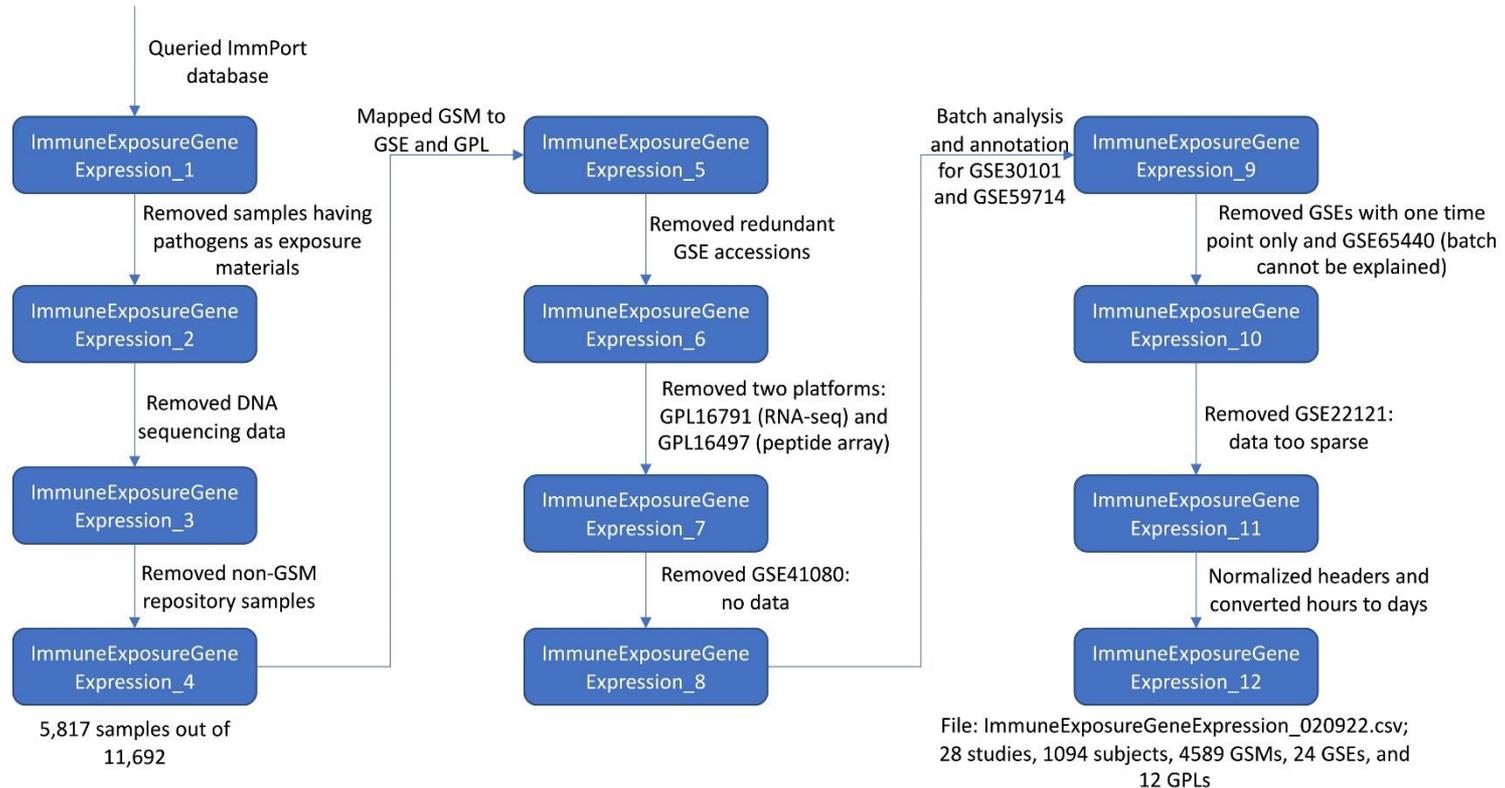
SPECIES	PROTEINS	COMPLEXES	REACTIONS	PATHWAYS
S. pombe	1772	1845	1494	822
S. cerevisiae	1925	1837	1574	817
D. rerio	8695	8525	7444	1686
X. tropicalis	7092	7392	6213	1589
G. gallus	7350	7991	6899	1714
C. familiaris	8221	8796	7514	1667
B. taurus	8905	9263	8123	1706
S. scrofa	8471	8902	7613	1670
R. norvegicus	8868	9582	8419	1712
M. musculus	9622	10794	9639	1725
*H. sapiens	11097	14277	14628	2629
D. melanogaster	4809	5446	4632	1487
C. elegans	4508	4432	3723	1317
D. discoideum	2782	2548	2328	991
P. falciparum	1147	1043	867	602

Supported by 36,600
PubMed indexed papers
manually annotated by
Reactome curators

Project Goals



Workflow to Collect Vaccination Metadata From ImmPort and Manual Annotation



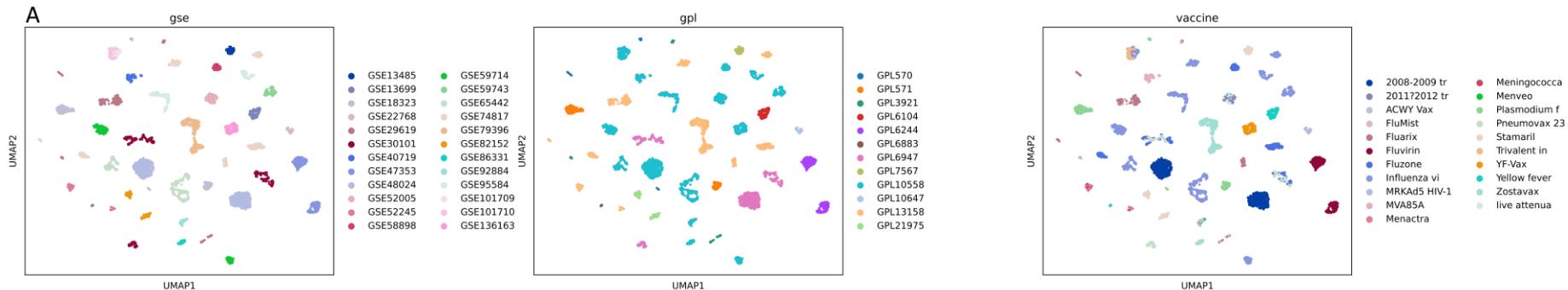
Statistics of the Vaccine Response Gene Expression Data Collected at VIGET

Object/Variable	Number/Value
Vaccine	21 (by names) or 20 (by VO ids)
ImmPort Study	28
Race	7
Min_Age	0
Max_Age	90
Min_Day	-7
Max_Day	84
Cell Type	4
Cell Subtype	184
Biosample (GSM)	4859
GSE	24
GPL Platforms	12

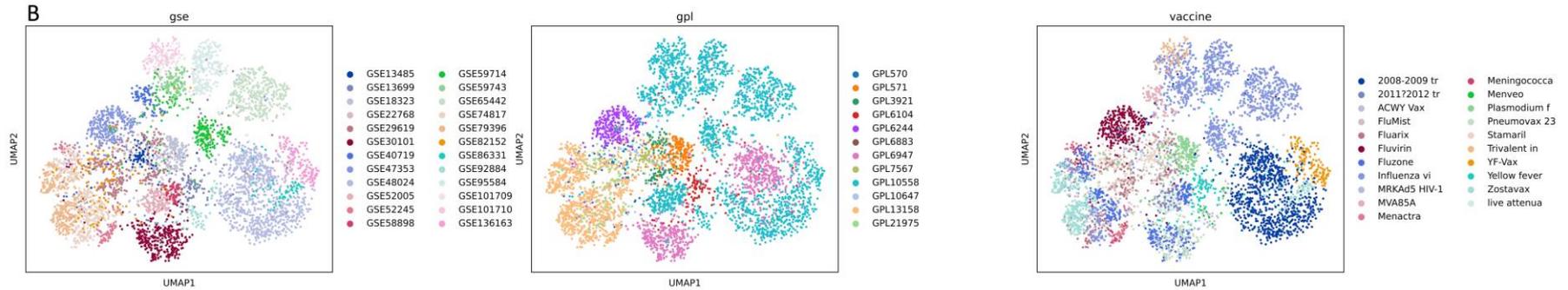
Vaccine Ontology Guided List

Vaccine	VO_ID	Category	Races	minAge	MaxAge	minDays	maxDays	Cell_Types	Cell_Subtypes	GSMs	GSEs	GPLs	Studies
2008-2009 trivalent influenza vaccine	VO_0004809	inactivated influenza vaccine	1	18.2	40.2	0	14	1	1	848	1	2	1
2011?2012 trivalent inactivated vaccine (A/California/7/09 (H1N1,), A/Perth /16/2009 (H3N2), and B/Brisbane/60/2008)	VO_0004810	inactivated influenza vaccine	3	0.5	13	0	30	1	1	64	1	1	1
ACWY Vax	VO_0003138	subunit vaccine	1	30	70	0	35	1	1	44	1	1	1
FluMist	VO_0000044	live attenuated influenza vaccine	3	0	47	0	7	1	5	127	1	2	1
Fluarix	VO_0000045	inactivated influenza vaccine	3	0	47	0	7	2	6	230	2	3	3
Fluvirin	VO_0000046	inactivated influenza vaccine	4	18	85	-7	70	1	176	292	1	1	1
Fluzone	VO_0000047	inactivated influenza vaccine	6	18	90	-7	28	3	1	417	3	3	3
Influenza virus vaccine	VO_0000642	viral vaccine	5	21	90	0	43	3	5	1189	6	2	5
MRKAd5 HIV-1 gag/pol/nef	VO_0003133	HIV Virus Vaccine	2	22	43	0	7	1	1	50	1	1	1
MVA85A	VO_0003120	Mycobacterium tuberculosis vaccine	1	18	55	0	7	1	1	96	1	1	1
Menactra	VO_0000071	Neisseria meningitidis vaccine	1	18	45	0	7	1	1	51	1	1	1
Meningococcal Polysaccharide Vaccine, Groups A & C, Menomune A/C	VO_0010725	Neisseria meningitidis vaccine	1	18	45	0	7	1	1	39	1	1	1
Menveo	VO_0001246	Neisseria meningitidis vaccine	1	30	70	0	35	1	1	15	1	1	1
Plasmodium falciparum vaccine	VO_0000087	malarial vaccine	1	18	65	0	79	1	1	254	1	2	1
Pneumovax 23 (USA)	VO_0000088	Streptococcal pneumoniae vaccine	3	22	49	-7	28	1	1	167	1	1	1
Stamaril	VO_0003139	Yellow Fever Virus Vaccine	2	18	45	0	28	1	1	196	2	2	2
Trivalent inactivated influenza	VO_0000642	inactivated influenza vaccine	3	22	90	0	41	1	1	79	1	1	1
YF-Vax	VO_0000121	Yellow Fever Virus Vaccine	1	19	49	3	84	1	1	144	1	1	1
Yellow fever 17D vaccine vector	VO_0000122	Yellow Fever Virus Vaccine	1	18	65	0	60	2	1	142	1	2	1
Zostavax	VO_0000124	Varicella-Zoster Virus Vaccine	6	25	79	0	7	3	1	344	2	2	3
live attenuated influenza vaccine	VO_0001178	Influenza Virus Vaccine	4	3	14	0	30	1	1	71	1	1	1

UMAP Plots of Samples: Batch Effect

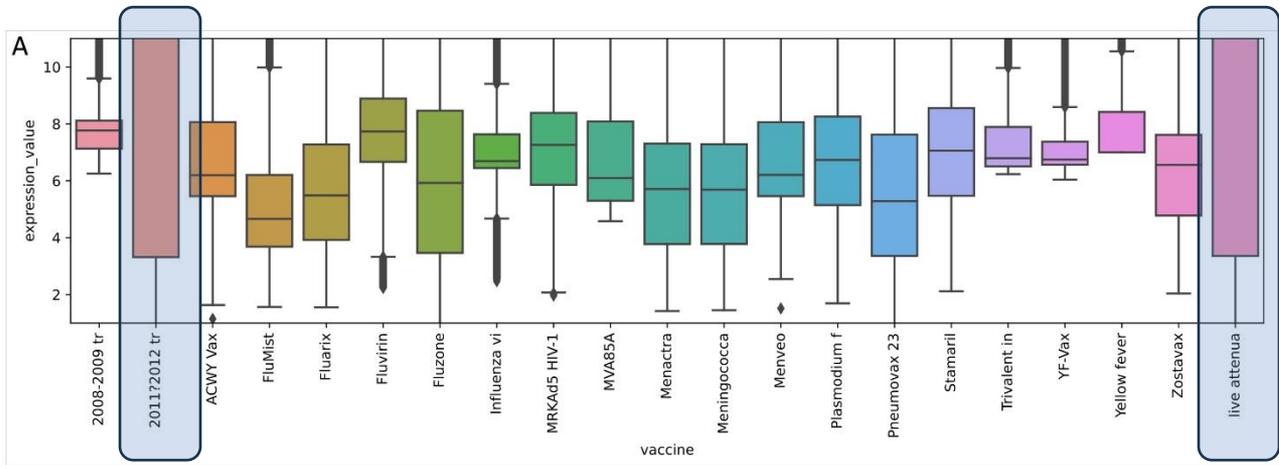


UMAP Plots of Samples: Batch Correction

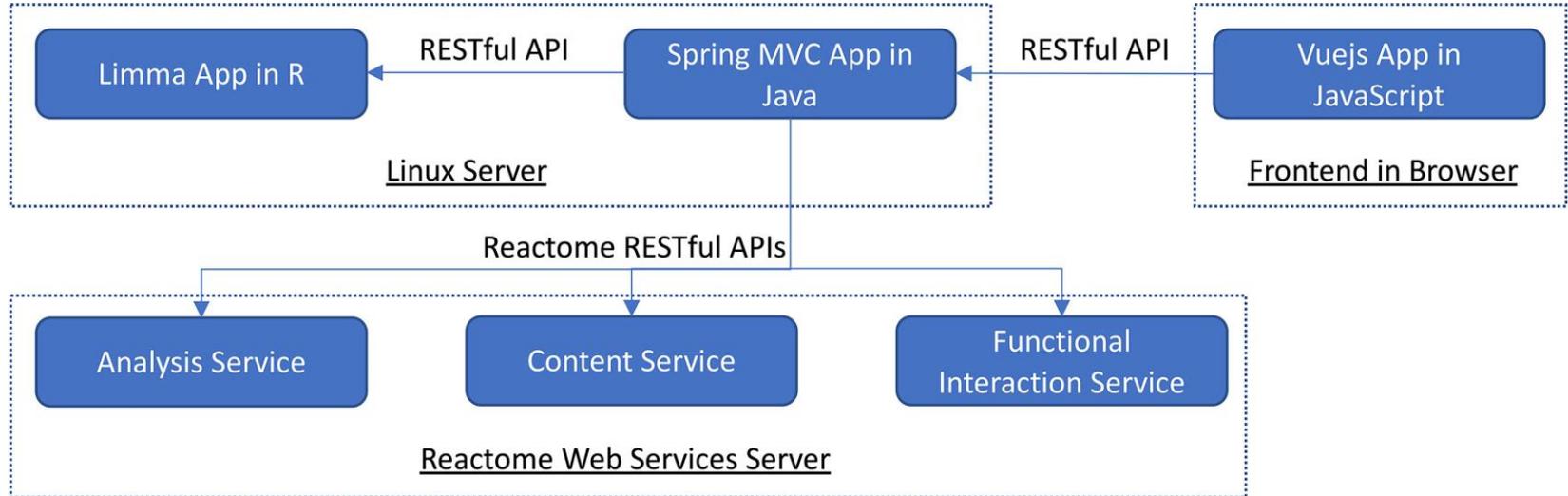


batch correction for GPL

Boxplot of Expression Values of Individual Vaccines



Software Architecture of VIGET



Two-step Protocol to Setup Differential Gene Expression Analysis for Vaccine Response @ VIGET

1 Filter Biosamples

2 Setup Analysis

- ▼ vaccine
 - ▼ inactivated vaccine
 - ▼ inactivated influenza vaccine
 - 2008-2009 trivalent influenza vaccine
 - Fluarix
 - Fluzone
 - 2011-2012 trivalent inactivated vaccine...
 - Fluvirin
 - live attenuated vaccine
 - live attenuated influenza vaccine

ImmPort Studies

- Select All
- [SDY269](#)
- [SDY270](#)
- [SDY61](#)

Platform Description

- Select All
- [HT_HG-U133A] Affymetrix HT Human Genome U133A Array
- [HT_HG-U133_Plus_PM]
- Affymetrix HT HG-U133+ PM

Day 0 Definition

- Select All
- Time of enrollment
- Time of initial vaccine administration

Gender

- Select All
- Female
- Male

Age

- 0.0 - 47.0

Race

- Select All
- Asian
- Black or African American
- White

Cell Type

- Select All
- PBMC : PBMC
- PBMC : CD11c hi CD123 lo mDC cells isolated from PBMCs
- PBMC : CD123 hi CD11c lo pDC cells isolated from PBMCs
- PBMC : CD14 + monocytes isolated from PBMCs
- Other : NA
- PBMC : CD19 + B cells isolated from PBMCs

CLEAR

NEXT

Two-step Protocol to Setup Differential Gene Expression Analysis for Vaccine Response @ VIGET

Filter Biosamples

2 Setup Analysis

① Create two groups for differential analysis by dragging samples from the first panel and then dropping them into Group 1 or Group 2 in the second panel

Time (days)	Samples	Time (days)	Samples	Other Options
7	26	Group 1	0 28	Adjust the analysis by checking the following variables: <input type="checkbox"/> Vaccine <input type="checkbox"/> Age <input type="checkbox"/> Gender <input type="checkbox"/> Race
		Group 2	3 26	Correct the results for: <input checked="" type="checkbox"/> Platform <input checked="" type="checkbox"/> Batch
				Use paired data by checking: <input checked="" type="checkbox"/> Paired
				Name the analysis result 3 vs 0

BACK

CLEAR

ANALYZE

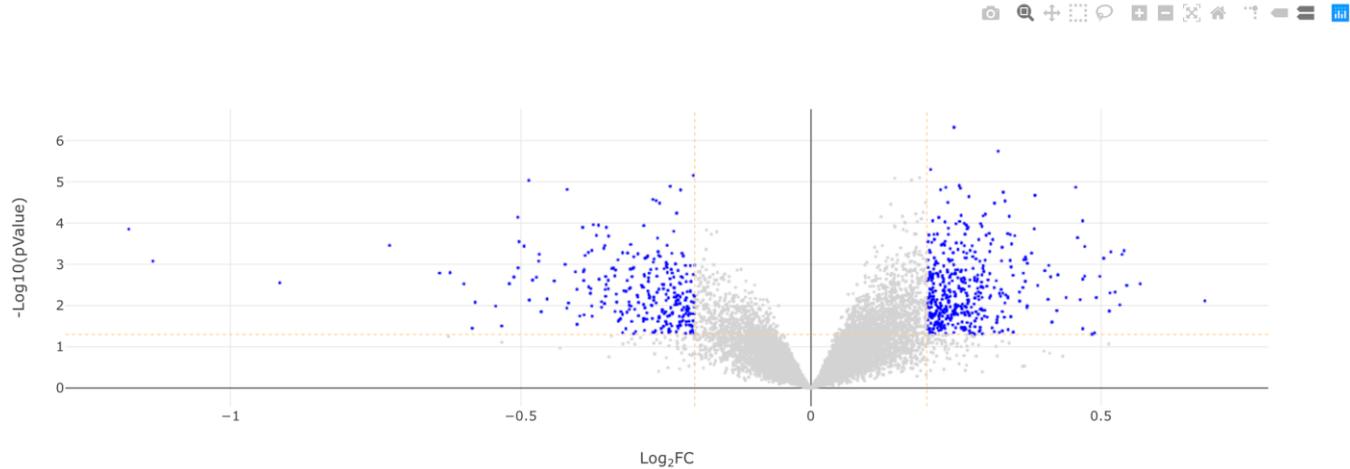
Analysis and Visualization Features @VIGET

Gene Expression Analysis

DOWNLOAD RESULTS

PATHWAY ANALYSIS

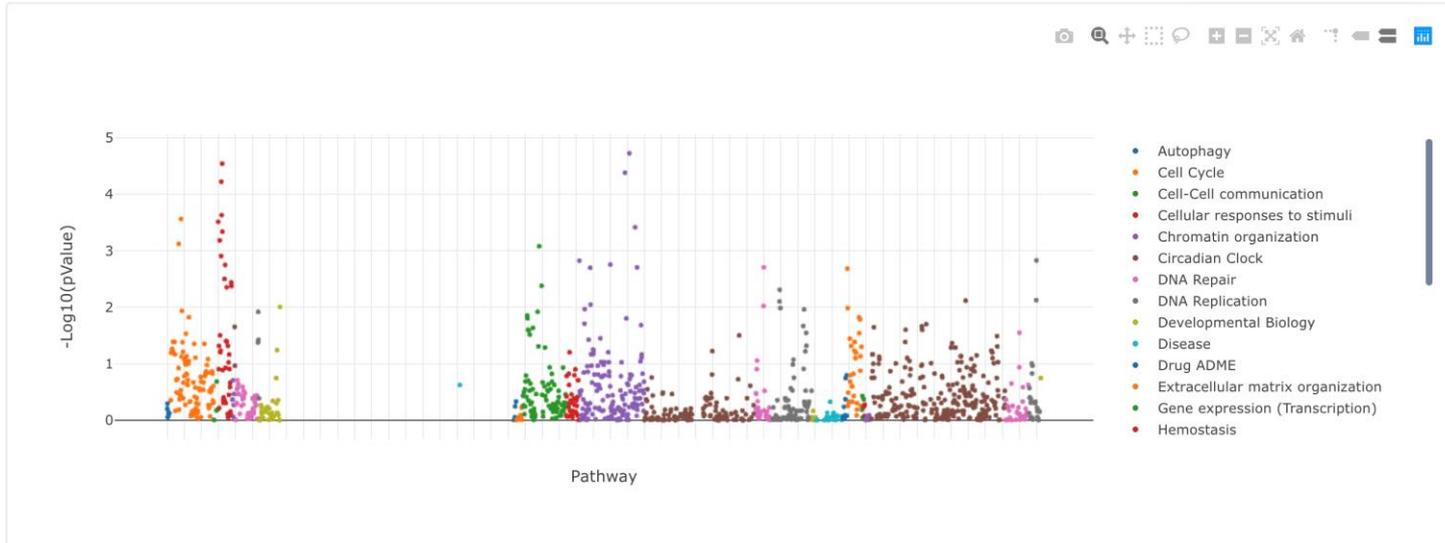
NETWORK ANALYSIS



Gene Name	Average Expression	Log ₂ FC	pValue	Adjusted pValue
PPP1R7	6.85e+0	2.47e-1	4.76e-7	8.96e-3
CBL	5.87e+0	3.23e-1	1.81e-6	1.70e-2
POLR2G	8.29e+0	2.07e-1	5.02e-6	1.84e-2
TRERF1	4.55e+0	-2.02e-1	6.98e-6	1.84e-2
RNF103	7.07e+0	-4.86e-1	9.19e-6	1.84e-2

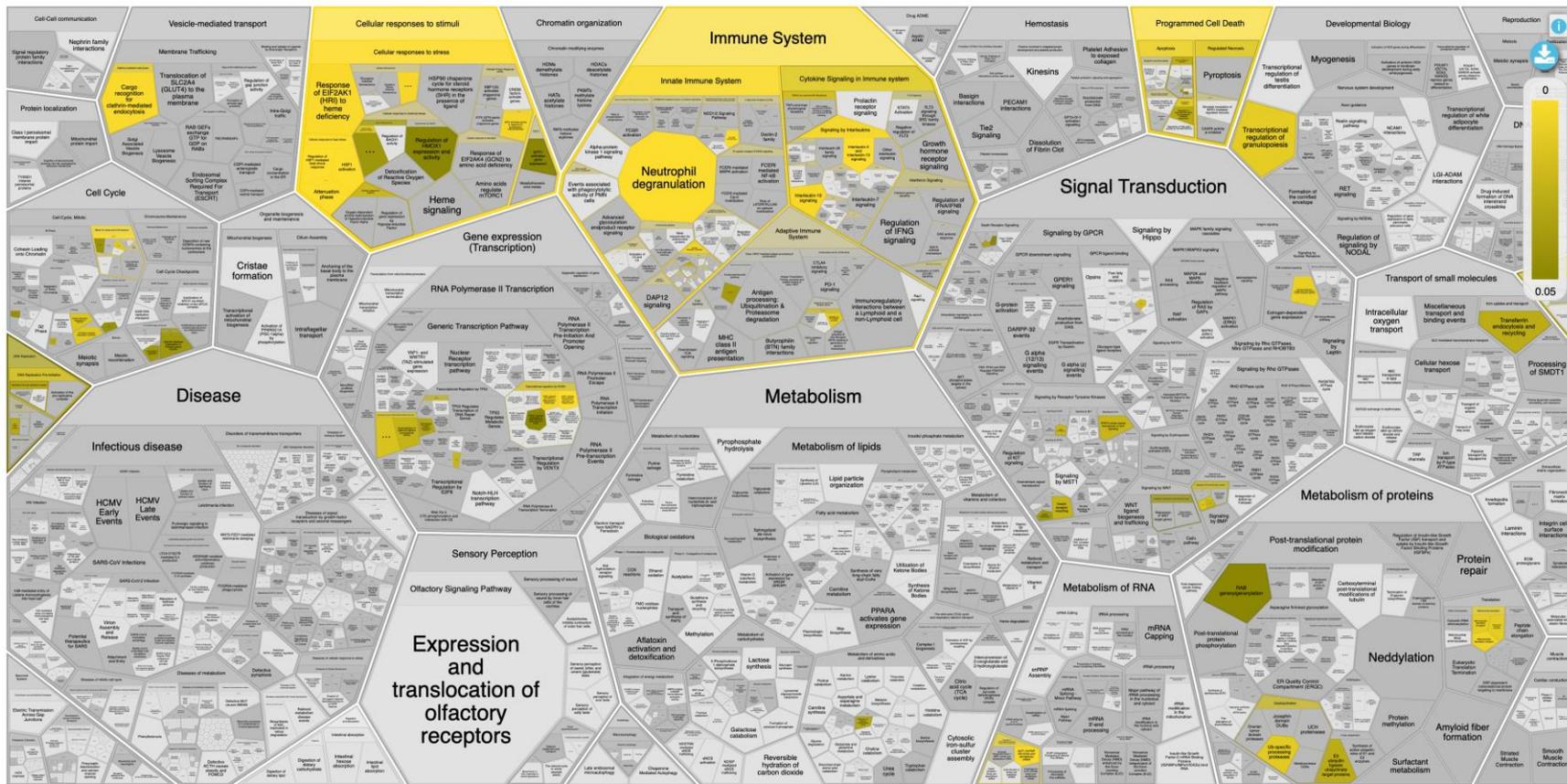
Analysis and Visualization Features @VIGET

Pathway Enrichment Analysis

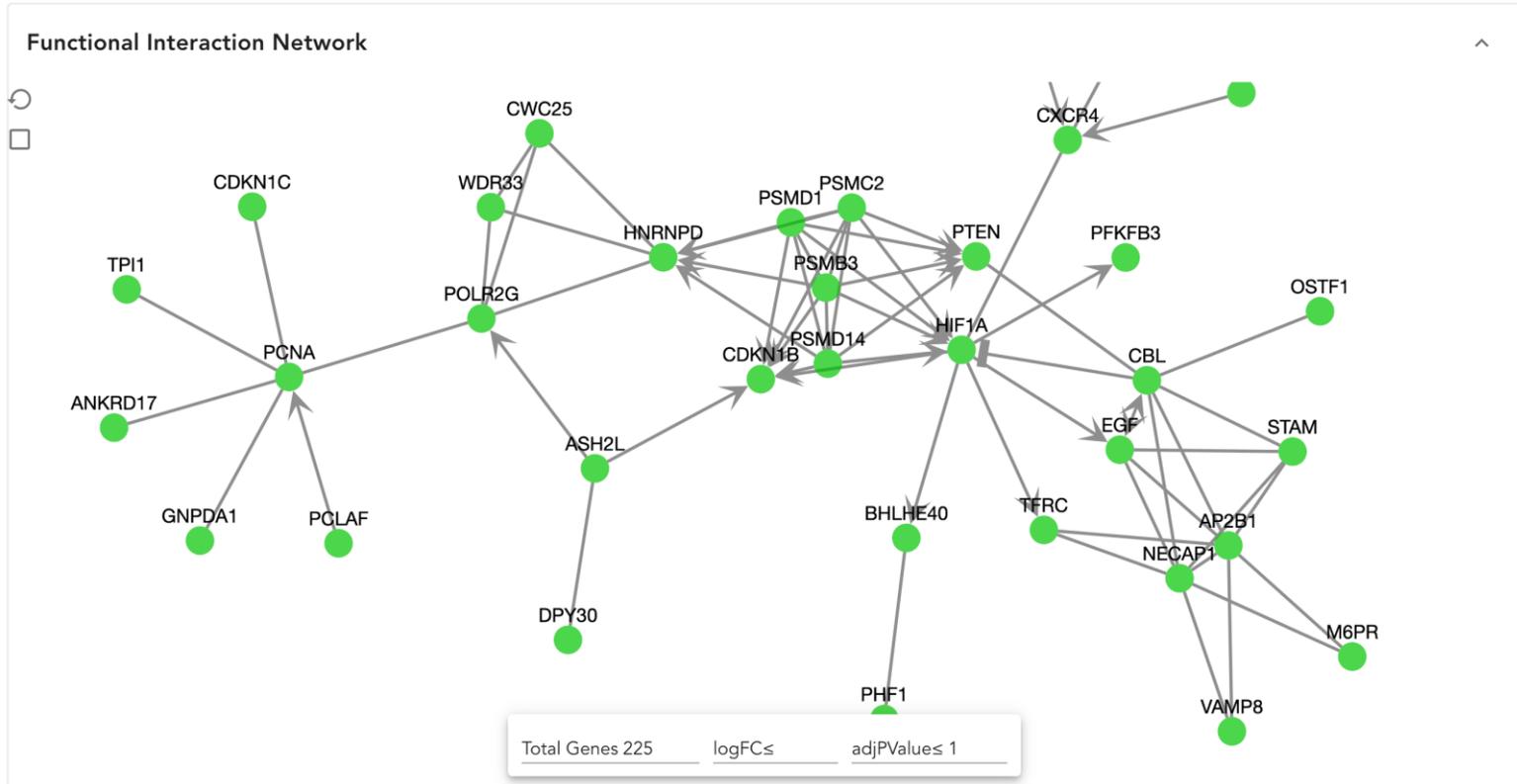
[DOWNLOAD RESULTS](#)[OPEN IN REACTOME](#)

Stable Identifier	Pathway Name	Entities Found	Entities Total	Entities Ratio	Entities pValue	Entities FDR
R-HSA-6798695	Neutrophil degranulation	51	480	3.17e-2	4.13e-5	2.05e-2
R-HSA-3371568	Attenuation phase	12	47	3.10e-3	2.86e-5	2.05e-2
R-HSA-449147	Signaling by Interleukins	66	658	4.35e-2	1.88e-5	2.05e-2
R-HSA-3371511	HSF1 activation	11	43	2.84e-3	5.97e-5	2.22e-2
R-HSA-3371571	HSF1-dependent transactivation	12	59	3.90e-3	2.34e-4	6.52e-2
R-HSA-69206	G1/S Transition	21	150	9.91e-3	2.73e-4	6.52e-2

Analysis and Visualization Features @VIGET



Analysis and Visualization Features @VIGET



Comparison Analysis Features @VIGET

3 vs 0 & 7 vs 3



Descriptions



3 vs 0 (a)

7 vs 3 (b)

Vaccines: Fluorix

Studies: SDY269

Platform Descriptions: [HT_HG-U133A] Affymetrix HT Human Genome U133A Array, [HT_HG-U133...

Day 0 Definitions: Time of enrollment, Time of initial vaccine administration

Genders: Female, Male

Ages: 0.0 - 47.0

Race: Asian, Black or African American, White

Cell Type: PBMC : PBMC

Study Variables: None

Platform Correction: Yes

Group 1 (Time: days): 0

Group 2 (Time: days): 3

Vaccines: Fluorix

Studies: SDY269

Platform Descriptions: [HT_HG-U133A] Affymetrix HT Human Genome U133A Array, [HT_HG-U133...

Day 0 Definitions: Time of enrollment, Time of initial vaccine administration

Genders: Female, Male

Ages: 0.0 - 47.0

Race: Asian, Black or African American, White

Cell Type: PBMC : PBMC

Study Variables: None

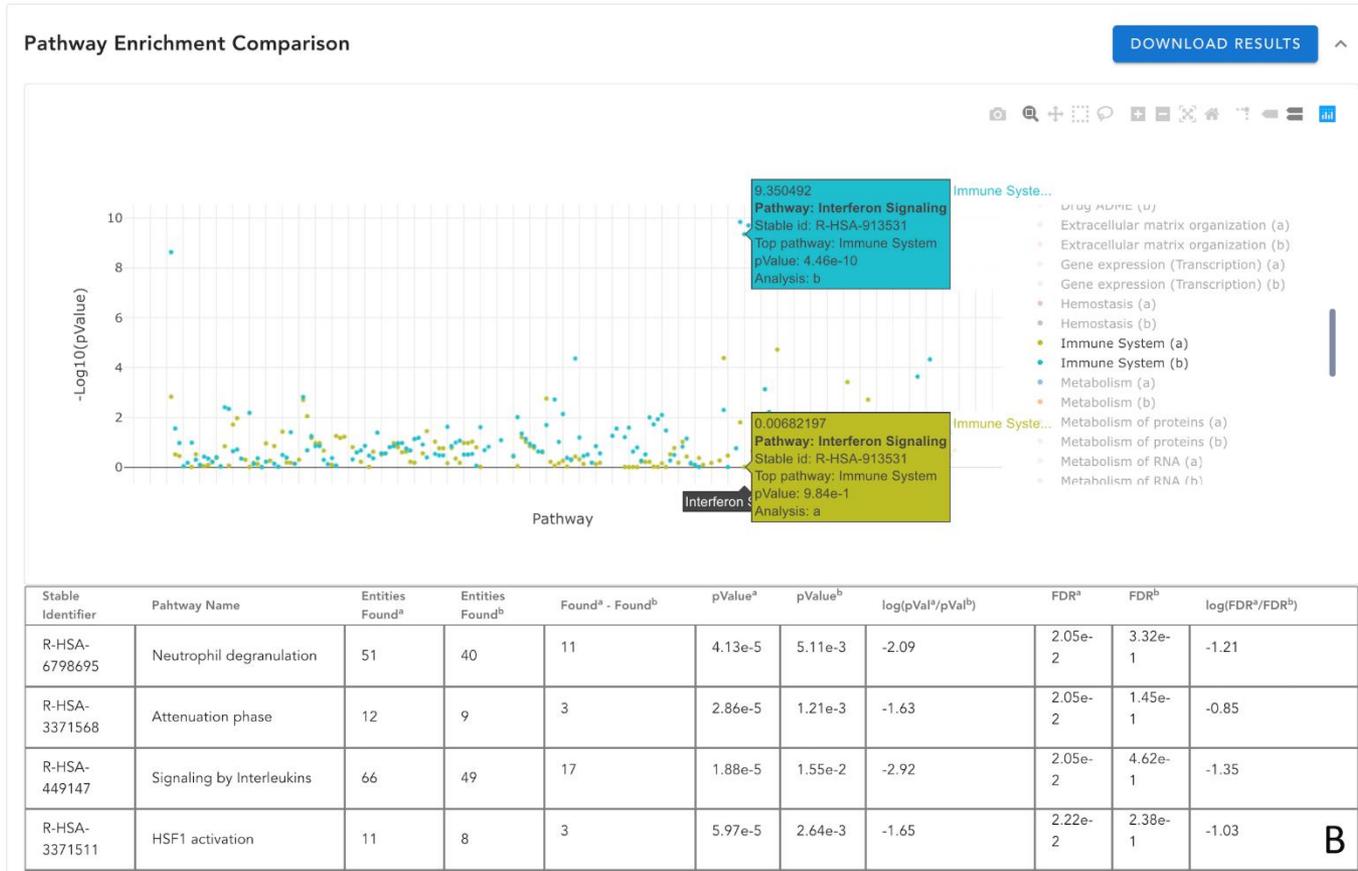
Platform Correction: Yes

Group 1 (Time: days): 3

Group 2 (Time: days): 7

A

Comparison Analysis Features @VIGET



Project Links

- Web site: <https://viget.violinet.org>
- Paper: Brunson T, Sanati N, Huffman A, Masci AM, Zheng J, Cooke MF, Conley P, He Y and Wu G (2023) VIGET: A web portal for study of vaccine-induced host responses based on Reactome pathways and ImmPort data. [Front. Immunol. 14:1141030](https://doi.org/10.3389/fimmu.2023.1141030). doi: 10.3389/fimmu.2023.1141030
- GitHub Repos
 - The source code for the web frontend app: <https://github.com/VIOLINet/reactome-immport-web>
 - The source code for the server-side application: <https://github.com/VIOLINet/immport-ws>
- Data: <https://doi.org/10.5281/zenodo.7407195>
 - ImmuneExposureGeneExpression_020922.csv: The meta file describing aggregated ImmPort studies and related vaccines, GEO information, and some manual annotation
 - Immport_vaccine_expression_matrix_mapped_merged_approved_genes_091421.csv: The aggregate gene expression data
- Open source and open access for both academic and commercial use: CC0 for data and CC BY 4.0 for Software

Software Demo

1. Perform a differential gene expression analysis
 1. Use Fluarix as an example
2. Conduct comparison analysis
 1. Fluarix response between 3 vs 0 days and 7 vs 3 days

Use VIGET to study vaccine responses to yellow fever vaccines

- Target: Longitudinal human responses to yellow fever vaccines
 - 4 ImmPort studies imported
 - 3 vaccines: YF-Vax, Stamaril, and Yellow fever 17D
 - Samples: 482, collected from whole blood or PBMCs.
 - Subjects: 18-65 years old, with Asian & African Americans, males & females.
 - Time: day 0 to day 84 post vaccination.
 - Focused: Immune profiles during the first 4 weeks post vaccination
- Reactome three categories of immune pathways focused:
 - Adaptive immune system
 - Innate immune system
 - Cytokine signaling in immune system
 - Reactome Immune system: <https://reactome.org/PathwayBrowser/#/R-HSA-168256>

Ref: Brunson T, Sanati N, Huffman A, Masci AM, Zheng J, Cooke MF, Conley P, He Y, Wu G. VIGET: A web portal for study of vaccine-induced host responses based on Reactome pathways and ImmPort data. *Frontiers in Immunology*. 2023. 14:1141030. PMID: 37180100 PMCID: [PMC10172660](https://pubmed.ncbi.nlm.nih.gov/37180100/).

Use VIGET to study vaccine responses to yellow fever vaccines

The screenshot displays the VIGET application interface, divided into two main sections labeled A and B.

Section A: This section contains several panels for configuring the study. The leftmost panel shows a list of vaccine types with checkboxes: Fluzone, 2011-2012 trivalent inactivated vaccine..., Fluvirin, live attenuated influenza vaccine, FluMist, Yellow Fever Virus Vaccine (checked), YF-Vax (checked), Stamaril (checked), and Yellow fever 17D vaccine vector (checked). Other panels include:

- ImmPort Studies:** Select All (checked), SDY1264 (checked), SDY1289 (checked), SDY1294 (checked).
- Platform Description:** Select All (checked), Human Genome U133 Plus 2.0, Custom CDF Version 9 (checked).
- Day 0 Definition:** Time of initial vaccine administration (checked).
- Gender:** Select All (checked), Female (checked), Male (checked), Not Specified (checked).
- Age:** Select All (checked), 18.0 - 45.0 (checked), 18.0 - 65.0 (checked), 19.0 - 49.0 (checked).
- Race:** Select All (checked), Asian (checked), Black or African American (checked).
- Cell Type:** Select All (checked), Whole blood : NA (checked), PBMC : NA (checked).

Section B: This section is for creating analysis groups. It includes a table of samples, group configuration boxes, and other analysis options.

Table:

Time (days)	Samples
5	5
3	96
10	15
14	59
21	6
28	33
60	9
84	36

Group Configuration:

- Group 1:** Time (days) = 0, Samples = 72
- Group 2:** Time (days) = 7, Samples = 114

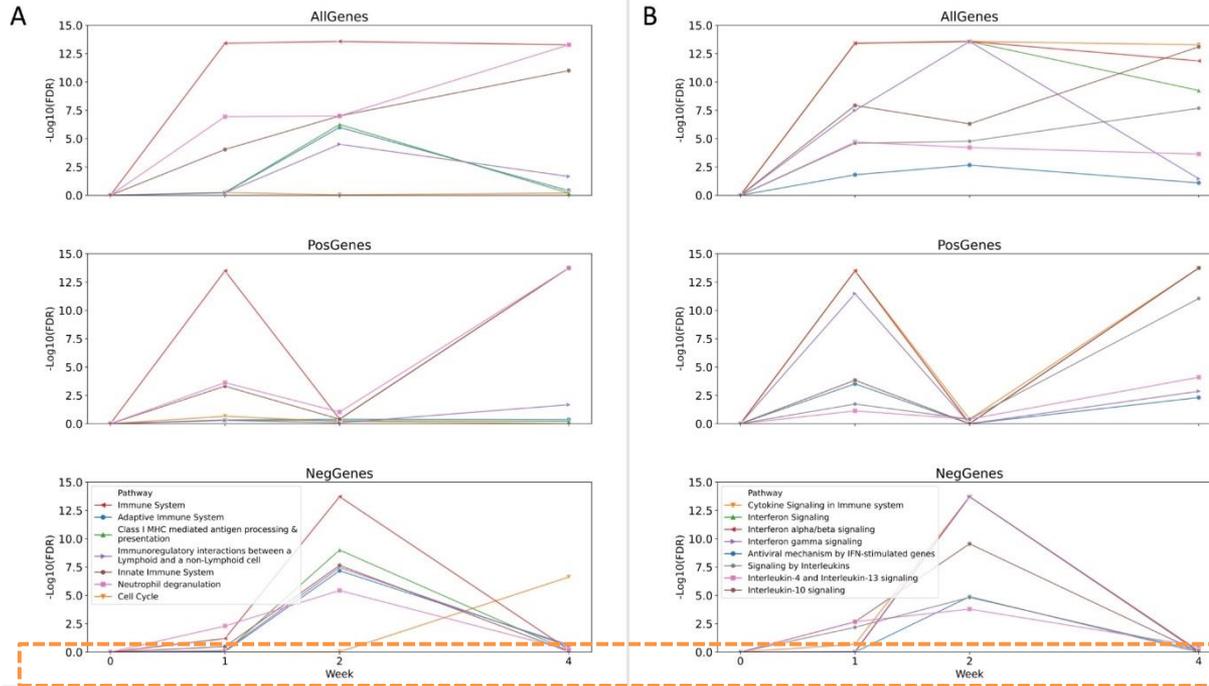
Other Options:

- Adjust the analysis by checking the following variables: Vaccine (checked), Age (checked), Gender (checked), Race (checked).
- Correct the results for: Platform (checked), Batch (checked).
- Use paired data by checking: Paired (checked).
- Name the analysis result: 0 vs 7 Days

Fig. 3

**Condition
Setup
For the
Use case**

Temporal Changes of Immune & Cytokine Signaling Pathways



Activated at 1 week:

- Interferon α/β
- Interferon γ
- Innate immunity
 - Neutrophil degranulation

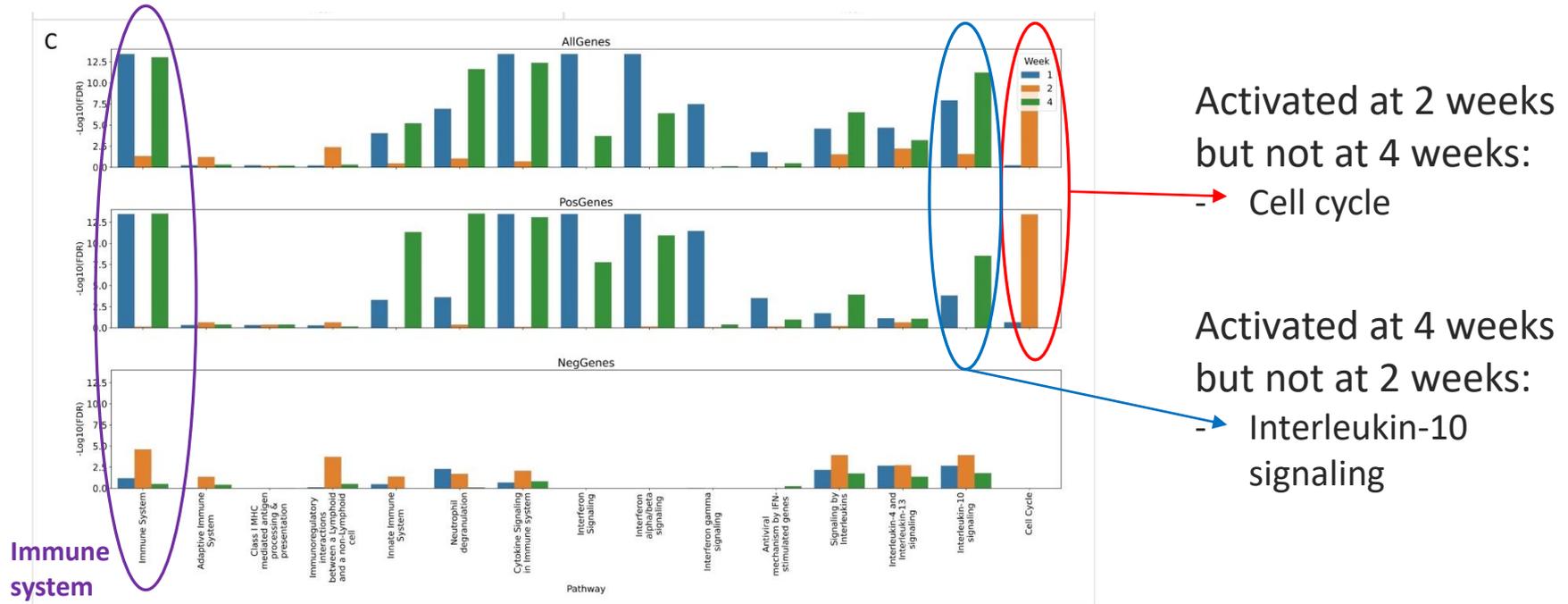
1st four weeks post vaccination

Temporal changes of major immune response pathways and cell cycle (Fig. 9A)

Temporal changes of cytokine signaling pathways (Fig. 9B)

Responses to yellow fever vaccines

Immune Response Pathways Enriched at 1-4 weeks post vaccination



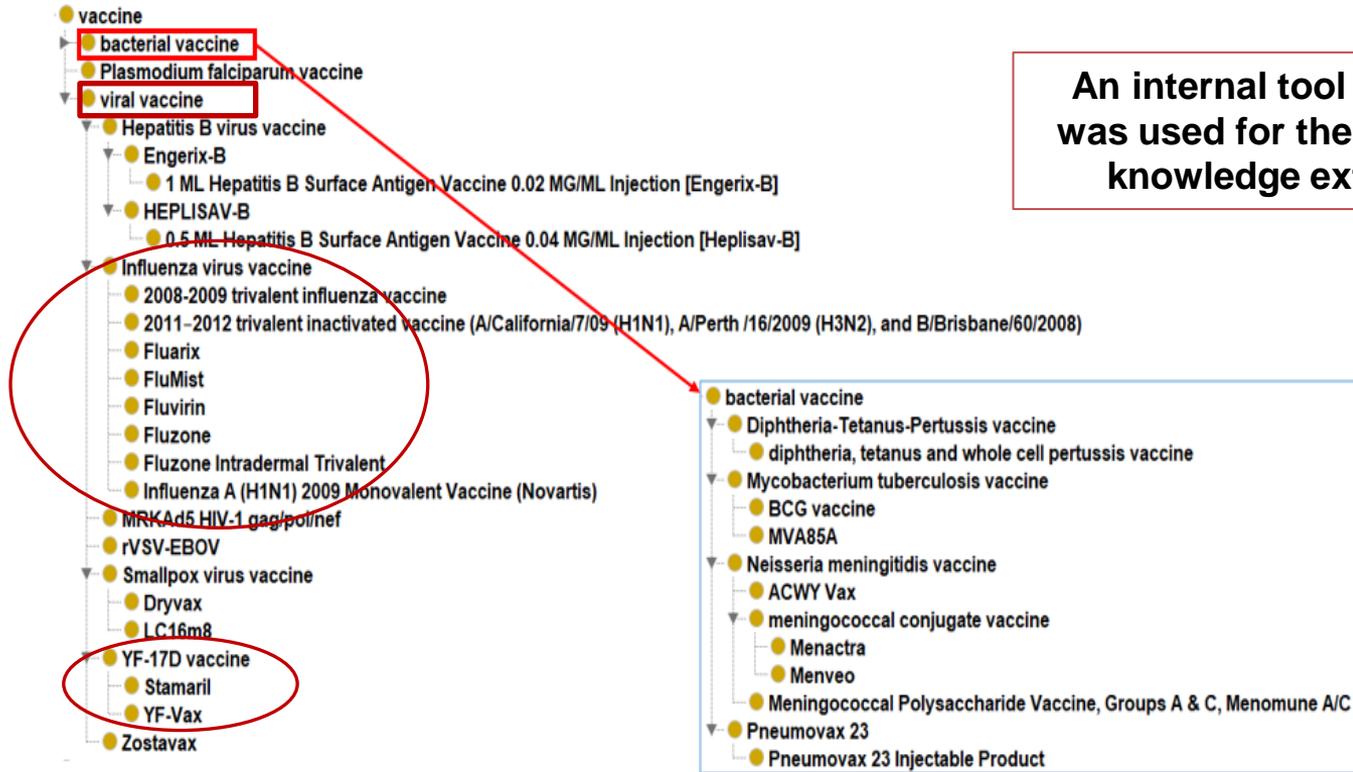
Immune response pathways enriched for genes significantly differentially expressed at 1, 2, & 4 weeks post vaccination (Fig. 9C)

Summary

- **VIGET**: A web portal to study vaccine responses based on Reactome pathways and ImmPort data
- Use case: VIGET was used for analysis of human responses to yellow fever vaccine

Discussion

Vaccine Ontology (VO) IDs used in ImmPort has been used to automatically build up hierarchical structures, improving analysis



An internal tool (Ontofox) was used for the automatic knowledge extraction

Future Work

- Possible inclusion of VIGET in ImmPort Resources
- Expand VIGET with more comprehensive and updated data
- Being used for more use cases
- Collaboration with ImmPort

The screenshot displays the ImmPort Resources website. At the top, there is a navigation bar with the ImmPort Resources logo and three main menu items: Publications, Documentation, and Tutorials. Below this, the 'Programs' section is divided into three columns. The first column contains AMP RA/SLE, HIPC, and SeroNet. The second column contains the NIH, NIAID, pharmaceutical companies and nonprofit organizations partnership (AMP) to develop new ways of identifying and validating promising biological targets for diagnostics and drug development. The third column contains the Bill & Melinda Gates Foundation, the purpose of the Bill and Melinda Gates Foundation and University of Cape Town tuberculosis collaboration is to identify prospective immune correlates of risk of tuberculosis (Tb) disease, and the March of Dimes Database for Preterm Birth Research. Below the 'Programs' section is the 'Additional Resources' section, which is also divided into three columns. The first column contains ImmuneSpace, ImmTransplant, Coronavirus Disease 19, and Gene Lists. The second column contains 10k Immunome, Cell Ontology Browser, immunEXpresso, and ImmPort API's. The third column contains the 10,000 Immunomes Project, an interactive visualization and query tool for the Cell Ontology, immunEXpresso (IX) allows to learn about reported interactions between various cell types and regulatory molecules (cytokines and chemokines), and Application Program Interface to query the shared data and a tool to download result files.

<https://www.immport.org/resources>

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