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# Introduction

Identifying the gene products that are differentially expressed is a key area for understanding essential mechanisms causing diseases such as cancer and autoimmune disorders (1). Understanding the biological pathways of the genes involved is also critical for the study and characterization of diseases in order to develop effective treatments.

Pathway Studio®, a product of Elsevier R&D Solutions, utilizes a knowledgebase of biological relationships developed from millions of articles and abstracts as well as clinical trials, to assist researchers in this important endeavor.

SoftGenetics' NextGENe software now provides a direct link into Elsevier's Pathway Studio for Pathway Studio subscribers, allowing seamless mining of biological systems. NextGENe software's Expression Comparison tool can be used to identify the differentially expressed genes and with a simple click of a button, NextGENe software's expression analysis results can be uploaded to Pathway Studio, enabling the visualization of the disease mechanisms, gene expression and more. This automated linkage can improve laboratory efficiency by allowing researchers to quickly access a broad knowledge base without requiring tedious and error prone manual searches.

In the example that follows NextGENe software was used to determine the expression levels in the Universal Human Reference (UHR) and the Human Brain Reference (HBR) samples. Differential expression analysis of these samples was conducted using Elsevier's Pathway Studio and the associated biological pathways, diseases and processes were reviewed for the differentially expressed genes.

# Procedure

### NextGENe Expression Comparison

- 1. Create a project for the control and a project for the sample with NextGENe software.
- Open the NextGENe Viewer's Process menu and select Pathway Studio Settings. Choose the appropriate Access option based on your subscription: (Mammalian/ChemEffect/DiseaseFX, Mammalian, or Plant).
- 3. Open the NextGENe software Viewer's Comparisons menu and select Expression Comparison Report.
- Select "Use segments as defined in reference files" and "Gene" as Regions to calculate expression statistics.
- Load the \*.pjt file for the control project as the Reference, and load the \*.pjt file(s) for the sample project(s) as Samples. Click "OK".
- 6. In the Expression Comparison Report window that opens, from the File menu, choose to "Upload gene list to Pathway Studio".





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Name	UHR_Output	Product	NextGENe			
Organization	Softgenetics	Organism	Human			
Property description		(Optional)				
Property source		(Optional)				
Sample						
Sample name	UHR_Output	Class name	strain	Value	control	
Sample name	HBR_Output	Class name	strain	Value	case	j
Differential expression	1					
DE1 Name	HBR_Output vs UHR_Output					
Sample set1	URD. Output	Sample set2				

7. Enter Sample Settings. The project results will be listed in Pathway Studio with the "Name" provided.

The Expression Comparison data is imported to Pathway Studio and differential expression values of log2ratio fold change and p-value are calculated. A list of all submitted projects is shown in Pathway studio. Double-click on any project to view these comparative values in table format. Follow the steps below to carry out a detailed differential expression pathway analysis.

#### Pathway Studio Differential Expression Analysis

- 1. Once the gene expression ratios are uploaded to Pathway Studio, right click on the Project and select Experiment Summary Analysis.
- 2. Click "Calculate Differential Expression", choose the Case and Control and Add them to the Differential Expression step. Optionally select a Multiple Testing Correction option. Click "Run".
- 3. Select the Differential Sample and choose "Start Analyses".

### **Results**

The Summary will show the Top Differentially Expressed Genes and all relevant pathways, diseases, cell processes and regulators. When evaluating the differential expression between the Universal Human Reference (UHR) and the Human Brain Reference (HBR), top diseases included epilepsy, top regulators included REST gene and top upregulated genes includes STX1B, as would be expected for a comparison of these samples.

Summary contains 15 results with 117 pathways and 2 groups	Top Diseases Name: Sub-Network	ks for 0202_UHR_HBR: c	ase vs control				
4 🗟 Summary	Name	Total # of Neighbors	# of Measured Neighbors	Gene Set Seed	Measured Neighbors	Median change	p-value
Top Pathways	Upstream Neighbors of Epilepsy	537	392	Epilepsy	KCNA2, SLC6A1, SN	2.11826	4.60117E-7
Immunological Pathways	Upstream Neighbors of Seizures	648	464	Seizures	KCNA2, SLC6A1, SN	1.55347	4.78916E-7
I oxicity Pathways	🗟 Upstream Neighbors of hippocampal sclero	18	14	hippocampal sclero	SLC6A1, MAPK8, KC	10.76527	1.68490E-5
Expression Targets Pathways	Upstream Neighbors of Status Epilepticus	236	170	Status Epilepticus	SLC6A1, AQP4, RIM	1.85014	2.16273E-5
Metabolic Pathways	Upstream Neighbors of Schizophrenia	545	399	Schizophrenia	SLC6A1, SNAP25, A	1.76872	2.40475E-5
Receptor Signaling	Upstream Neighbors of seizure susceptibility	114	79	seizure susceptibility	AQP4, GNG3, SLITR	2.85061	5.36981E-5
Cell Signaling	🔊 Upstream Neighbors of hemiplegic migraine	7	7	hemiplegic migraine	ATP1A2, ATP1A3, SL	52.96491	1.71737E-4
Inflammation Pathways	Upstream Neighbors of Hyperkinesis	41	27	Hyperkinesis	SNAP25, HTR2A, GR	7.28571	1.78881E-4
Signal Transduction Pathways	🗟 Upstream Neighbors of Pulmonary Embolism	41	27	Pulmonary Embolism	FABP3, APLN, ELAN	-4.57475	1.99700E-4
Disease Collections	Upstream Neighbors of malignant ascites	21	11	malignant ascites	FLT1, KDR, CD9, VE	-8.58462	2.07986E-4
Cell Process Pathways							
Diseases							
Iop Cell Processes							
<ul> <li>Image: Second Sec</li></ul>							

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## Discussion

Utilization of the NextGENe software and Pathway Studio combination provides researchers with a quick powerful tool which provides significant research efficiency. Differential expression analysis values as well as biological pathway information can be accessed with a simple workflow and can be easily navigated to review genes, diseases and pathways through a detailed visualization.

From any report, you can also view the pathway for genes in Pathway Studio by selecting a gene or list of genes, right-clicking on the selection and choosing to View in Pathway Studio.



NextGENe software also provides user-friendly solutions for other NGS applications such as variant detection, targeted sequencing, copy number variation analysis and HLA typing. In addition to gene expression analyses, Pathway Studio can also be used with NGS variant analysis datasets to evaluate variants' known associations with genes, diseases, cell processes and drug interactions. Pathway Studio subscription can be purchased through Elsevier R&D Solutions. (https://www.elsevier.com/solutions/pathway-studio-biological-research)

## Reference

 Gruden K, Hren M, Herman A, Blejec A, Albrecht T, Selbig J, Bauer C, Schuchardt J, Or-Guil M, Zupancic K. et al. A "crossomics" study analysing variability of different components in peripheral blood of healthy caucasoid individuals. PLoS One. 2012;7(1):e28761. doi: 10.1371/journal.pone.0028761.



